

A new species of *Cypris* (Crustacea: Ostracoda) from the Iberian Peninsula and the Balearic Islands, with comments on the first ostracod named using the Linnean system

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Abstract

The genus *Cypris*, considered the oldest ostracod generic name erected using the Linnean system, comprises a reduced number of large-bodied species, mostly found in Africa and Asia. Only six of them are known to occur in Europe. Here we describe a new species, *Cypris pretusi* **sp. nov.**, collected in small temporary streams and ponds along the Eastern Iberian Peninsula and Minorca (Balearic Islands). The new species is very close to the type species of the genus, *Cypris pubera* O.F. Müller, 1776, but differs from it in having a set of smaller subequal spines on the posterior edge of the valves, by the absence of conspicuous spines along the front edge, and by the beak-like frontal shape of its carapace in dorsal view, similar to *Cypris decaryi* Gauthier, 1933. Soft parts are very similar to the type species, but it differs in having shorter swimming setae on the second antennae. Molecular analyses of the COX1 region support its status as a species distinct from *C. pubera* and closer to *Cypris bispinosa* Lucas, 1849, also providing evidence for a separation of *C. pubera* s.l. in two clades, one of which is here considered to correspond to *Cypris triaculeata* Daday, 1892. We discuss the relationships of *C. pretusi* **sp. nov.** to other members of the genus and its possible origin from nearby biogeographic regions (probably Africa or Asia) and provide a key to species of *Cypris* found in Europe. We also discuss the relationship between *Monoculus concha pedata* (= *M. conchaceus*), the first ostracod named by Linnæus, and *Cypris pubera*, the type species of the genus, described by Müller in 1776 and considered by him the same species as the one first named by Linnæus.

Key words: Ostracoda, *Cypris*, integrative taxonomy, distribution, identification key, new species

Introduction

The ostracod genus *Cypris* includes the largest species of freshwater Ostracoda typically found in Western Europe (Meisch 2000), although biogeographies are changing fast, and some other large(r) exotic genera can be found now in the area. Taking its big size into account, it is perhaps not surprising that the type species of this genus was arguably the first ostracod to be named using the Linnean system. Indeed, it was actually Linnæus (1748) himself who included the ostracod *Monoculus concha pedata* in the order of apteran insects in the 6th edition of his *Systema Naturæ*. He had already described this species in his *Fauna Svecica* (Linnæus 1746), providing the descriptive name of “*Monoculus antennis capillaceis multiplicibus, testa bivalvi*”, and its code, *Fn. 1185* (Fig. 1), is clearly referred to in his 1748 monograph (Fig. 2) (see also Baird 1837 and Karanovic 2012). However, in the 10th edition of his most famous work, Linnæus (1758) renamed the same species as *Monoculus conchaceus* (Fig. 3). Eventually, Müller (1776) renamed it as *Cypris pubera* O.F. Müller, 1776 (Fig. 4), which is at present recognized as the type species of the genus. Later on, Baird (1837) discussed in detail about these and other synonyms, together with the difficulties in being sure about which species each author was referring to, or the confusions among their works (e.g. some species with the same name look very different according to each author's drawings, and some descriptions do not

allow species discrimination), but that is another complex and long story that surely affects many of the present-day recognized taxa. The generic name *Monoculus* was eventually suppressed by the International Commission of Zoological Nomenclature (ICZN 1954), after the proposal raised by H. Munro Fox.

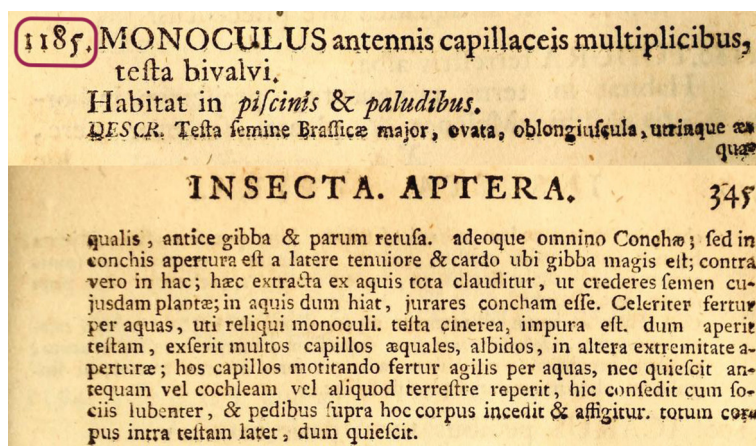


FIGURE 1. Bottom part of page 344 and top part of p. 345 of Linnæus' (1746) *Fauna Svecica*, including the description of an ostracod of the genus *Monoculus* with code 1185.

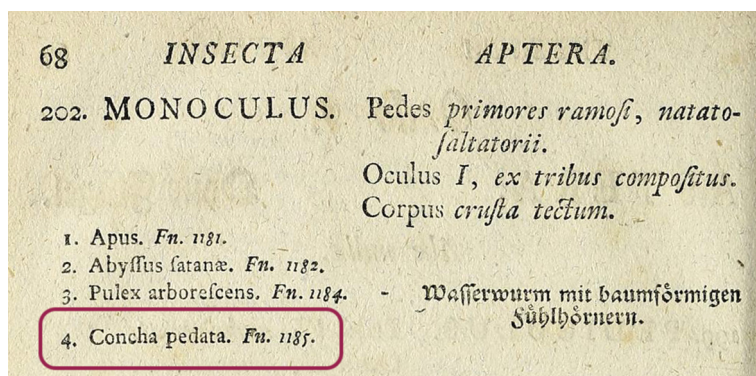


FIGURE 2. Top of page 68 of Linnæus' (1748) *Systema naturæ*, naming the first ostracod *Monoculus concha pedata*, and refer-
ring to his *Fauna svecica* code Fn. 1185 (see Fig. 1)

According to the most recent checklist of freshwater ostracods of the world by Meisch *et al.* (2019), there are at present 16 valid species in the genus *Cypris*, some of them with an uncertain taxonomic status. Out of these, only 6 are known from the Palaearctic Region: *Cypris pubera*, *Cypris granulata* Daday, 1898, *Cypris bispinosa* Lucas, 1849, *Cypris decaryi* Gauthier, 1933, *Cypris striata* (Jurine, 1820), and *Cypris maculosa* Bronstein, 1925. The highest species richness of this genus is found in the Afrotropical and Oriental regions. Karanovic (2012) had considered that there are 24 known species in the genus and Martens *et al.* (2013) had listed 21 species, but many of these were later discarded as dated or uncertain (Meisch *et al.* 2019) or as junior synonyms of other species, as in the case of *Cypris triaculeata* Daday, 1892 (considered a synonym of *C. pubera*). Therefore, it is still a dynamic genus in terms of the establishment of a clear understanding of its diversity, and its taxonomy seems to be far from settled. For instance, the species *C. striata* is known only from the type locality in Switzerland and from a second site in Budapest (Daday 1900), but its generic assignment is doubtful according to Meisch (2000). The drawings and description of this species by Jurine (1820), where he noticed a pattern of concentric lines on the valves similar to growth annuli of bivalves (as also described later by Desmarest 1825), and a carapace size smaller than that of *Cypridopsis vidua* (Jurine, 1820), which measures approximately 0.6 mm, suggest these could actually belong to juvenile individuals of *Cypris*, rather than adults, and notably differ from the much larger shell with straight lines drawn across the valves by Daday (1900), and redrawn in Meisch (2000). Indeed, Fischer (1851) already considered that *C. striata*, as described in Jurine (1820), actually corresponded to juveniles of *C. pubera*. Consequently, there are some founded doubts about the species described by Jurine (1820) as being the same as '*Cypris striata*' *sensu* Daday (1900), as suggested by Meisch *et al.* (2019).

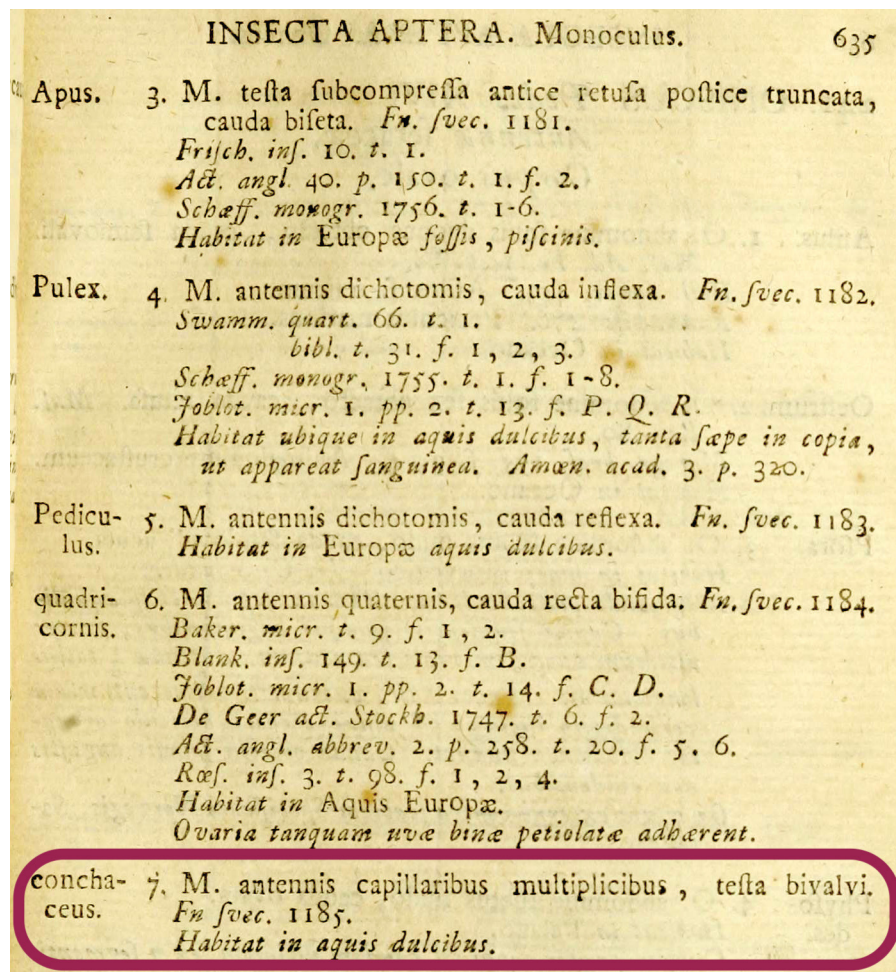


FIGURE 3. Top part of page 635 of Linnaeus' (1758) *Systema naturæ*, where he replaces the ostracod name *Monoculus conchapedata* by the new *Monoculus conchaceus*, referring to his *Fauna svecica* code *Fn Svec. 1185* (see Figs. 1–2)

Cypris bispinosa was the first member of the genus known to occur in the Iberian Peninsula, while *Cypris granulata* (as *C. subglobosa*) and *C. pubera* were found in this area by the late 20th century (Baltanás *et al.* 1996). Later on, Zamora *et al.* (2005a) found *Cypris decaryi* for the first time in the Balearic Islands. However, a recent reanalysis of similar specimens collected in the Iberian Peninsula, initially considered to belong to the same species as those found in the Balearic Islands, made the authors aware of some noticeable morphological differences with *C. decaryi* (as shown in Gauthier 1933 and Martens 1990), and therefore those specimens were left in open nomenclature as *Cypris* sp. by Castillo-Escrivà *et al.* (2016). A careful inspection of these specimens confirmed that in both cases they did not belong to either *C. decaryi* or any other described species of the genus *Cypris*, and consequently we decided to describe the new species, as detailed below.

Material and methods

The material analyzed for the description of the new species comes from four different campaigns (Table 1). The first one was carried out by one of us (JR) in May 2003, collecting some ostracods in the Palància river when studying its macroinvertebrate fauna. Soon after, on June 5th the same year, Prof. Joan Ll. Pretus (Univ. Barcelona) sampled a pond in the Natural Park s'Albufera des Grau (Minorca, Balearic Islands) and later delivered us a sample of selected ostracods for identification. The third campaign was done on the 25th of June 2004 in the headstreams of Palància river (Eastern Iberian Peninsula); these samples were collected by FMJ and JR. Finally, FMJ and JR collected fresh individuals from this river again in March 2019 for molecular analysis. In all cases, benthic samples were collected with hand nets, and fixed in the field with ethanol 70%. At the same time, environmental data were

obtained in some cases, including water electrical conductivity and pH in the samples from Minorca, and also water temperature and dissolved oxygen content in the third set of samples. In addition, a few individuals of *Cypris bispinosa* were collected in April 2019 from an isolated pond in Benirrama (Eastern Spain) and some individuals of *Cypris pubera* from a lateral pond in the Ebo river (Beniarbeig, near site EB4-P in Zamora *et al.* 2005b, where it had previously been found). These individuals were used for molecular analyses, in order to compare the COX1 sequences of these taxa with DNA from the new species (see below).

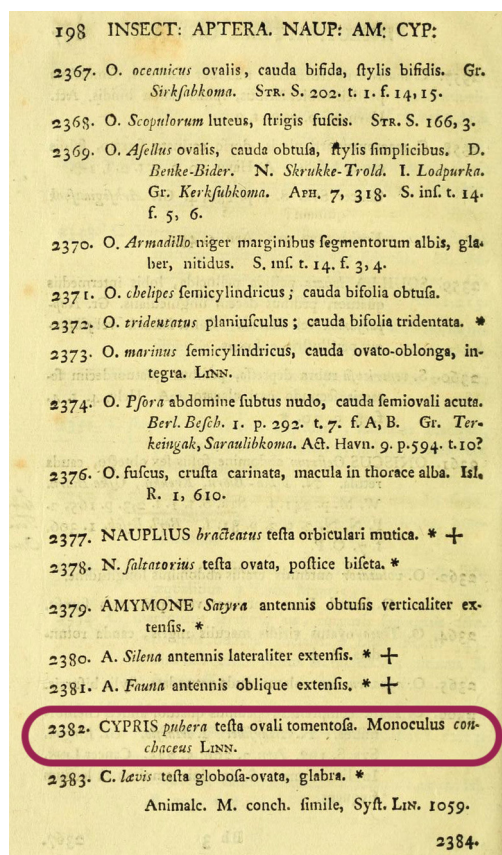


FIGURE 4. Page 198 of Müller (1776), where he introduces the ostracod species name *Cypris pubera* for the first time and synonymizes it with Linnaeus' *Monoculus conchaceus*.

A few ostracod individuals were directly isolated in the field from material deposited on a tray and using a Pasteur pipette. All other ostracod individuals were sorted out from net samples in the laboratory under a stereomicroscope, and identified to species level following mostly Meisch (2000). Ostracod dissections closely followed Namiotko *et al.* (2011); specimens with the valves open were placed in a drop of glycerin and the soft parts were dissected using fine needles. These body parts were then placed in a microscope slide and submersed in Hydromatrix® for permanent preparations, or kept in glycerin and the cover slide sealed with nail polish. The valves were cleaned in ethanol using a fine brush and, after drying, placed in a micropaleontological slide or in a SEM stub. SEM pictures were taken using a Philips XL-30 microscope at the University of Valencia. Nomenclature for soft parts anatomy and chaetotaxy follows mainly Broodbakker and Danielopol (1982), Martens (1987) and Meisch (2000), as used in Aguilar-Alberola and Mesquita-Joanes (2013), except that here we do not follow Smith & Tsukagoshi (2005) for the podomeres of the antennula to avoid confusion with more widely accepted segmentation codification, which can be more clear in the absence of ontogenetic information. Abbreviations used in the text and figures for valves, limbs and soft parts, include the following: Cp=carapace; H=height; L=length; W=width; LV=left valve; RV=right valve; An1=antennula; Ro=Rome organ; An2=antenna; Md=mandibula; Md-p=mandibular palp; Mx=maxillula; Mx bp=maxillular branchial plate; L5=5th limb; L6=walking leg; L7=cleaning limb; CR=caudal rami.

In order to put the new finding in the context of the distribution of other congeneric species, we built a provisional distribution map of *Cypris* species in Europe using QGIS 3.4.1 (QGIS Development Team, 2018). We considered *C. triaculeata* as a valid species (see discussion below), and therefore we included it separately from *C. pubera* in the distribution map. Distribution data was gathered from various sources, including databases such

as NODE (Horne *et al.* 1998) and GBIF (GBIF.org 2018), own unpublished data, and numerous published documents and reviews, in particular the works by Gauthier (1928), Fuhrmann & Goth (2011), Karan-Žnidaršič & Petrov (2007), Pieri *et al.* (2015), Curry *et al.* (2016), Özuluğ *et al.* (2018), Ghaouaci *et al.* (2017), Marrone *et al.* (2019), and references included in these works. The compilation is not complete, but has been built focusing on attaining a large-scale knowledge of the distribution of the genus in Europe, trying to avoid leaving large areas uncovered.

TABLE 1. Information on sampling sites where *Cypris pretusi* **sp. nov.** was collected. EC=water electrical conductivity, WT=water temperature, O₂=dissolved oxygen concentration, N= total number of individuals collected, IGI=*Ilyocypris gibba* (Ramdohr), CBI=*Cypris bispinosa*, CHA=*Cypridopsis hartwigi* G.W. Müller, SAC=*Sarscypridopsis aculeata* (Costa), SLA=*Sarscypridopsis lanzarotensis* (Mallwitz), IIN=*Ilyocypris inermis* Kaufmann, PAL=*Pseudocandona albicans* (Brady), PSP=*Pseudocandona* sp., HSA=*Heterocypris salina* (Brady), PVI=*Potamocypris villosa* (Jurine), HNT=*Herpetocypris intermedia* Kaufmann, HBR=*Herpetocypris brevicaudata* Kaufmann, (†)=only valve remains

SITE	Municipality	Habitat	Latitude (°)	Longitude (°)	Altitude (m)	Date
R. Palància	Teresa	Stream	39.899	-0.659	616	11/5/03
Bassa s'Enclusa	Maó	Pond	39.985	4.232	26	5/6/03
Rambla de Orduña	El Toro	Stream	39.971	-0.716	844	25/6/04
R. Palància	Bejís	Stream	39.930	-0.735	791	25/6/04
R. Palància	Bejís	Stream	39.930	-0.735	791	15/3/19
R. Palància	Teresa	Stream	39.899	-0.659	616	25/6/04

TABLE 1. (Continued)

SITE	EC (μS/cm)	WT (°C)	O ₂ (mg/L)	pH	N	Accompanying species
R. Palància	-	-	-	-	1	HBR, SLA, PSP
Bassa s'Enclusa	3300	-	-	7.59	19	IGI, CBI, CHA, SAC, SLA (†)
Rambla de Orduña	480	14.5	9.01	8.19	70	IIN, PAL, HSA, CHA, PVI
R. Palància	458	15.4	9.34	8.36	6	HNT, PVI
R. Palància	505	-	-	8.20	14	-
R. Palància	510	16.7	9.3	8.44	2	HBR, PVI

DNA extraction and COX1 sequencing. Total genomic DNA extraction was performed using the DNAeasy kit (QIAGEN, Valencia, California) following the manufacturer instructions. A new set of primers, covering the standard DNA barcoding region of the cytochrome oxydase subunit I (COX1), was specifically designed for the amplification of the COX1 gene in oligostraca species, COX1-OligoF: 5'-GRYTTTWTYTCYACRAATCAYAAR-GAYATTGG-3' and COX1-OligoR: 5'-AATTAARATRTADACTTCWGGRTGACC-3'. The polymerase chain reaction (PCR) thermal profile used was 94°C for 4 min for initial denaturation, followed by 30 cycles of 94°C for 30 s, 50°C for 30 s, 72°C for 30 s and a final extension at 72°C for 4 min. Amplified PCR products were purified with QIAquick PCR Purification Kit (QIAGEN Inc.) before direct sequencing of the product on an ABI Prism 3770. The chromatograms for each DNA sequence were checked using the software BioEdit ver. 7.2.5 (Hall 1999). All sequences were translated to amino acids to detect insertions, deletions and/or in-frame stop codons in order to discard presence of pseudogenes (Beltrà *et al.* 2015).

Sequence alignment was conducted using the program Muscle v3.6 (Edgar 2004) with default parameters. The molecular analysis involved 45 nucleotide sequences, including previously published sequences of *Cypris* (Subfamily Cypridinae) and using *Strandesia* species (Subfamily Cypricercinae) as the outgroup (see Fig. 8). The nucleotide substitution model with the lowest AICc value (Akaike Information Criterion, corrected) and the evolutionary history and phylogenetic position of *C. pretusi* **sp. nov.** were inferred by using the Maximum Likelihood method as implemented in MEGA 7 (Kumar *et al.* 2016). Initial trees for the heuristic search were obtained by applying the Neighbour-Joining method to a matrix of pairwise distances estimated using the Maximum Composite Likelihood approach. The Kimura 2-parameter (K2P) genetic distances and the corresponding standard deviations were also estimated between the newly obtained molecular data and sequences of *Cypris* species available in GenBank using MEGA 7 (Kumar *et al.* 2016).

Results: taxonomic description, phylogenetic analysis and ecological information

Class Ostracoda Latreille, 1806

Subclass Podocopa G.W. Müller, 1894

Order Podocopida Sars, 1866

Suborder Podocopina Sars, 1866

Superfamily Cypridoidea Baird, 1845

Family Cyprididae Baird, 1845

Subfamily Cypridinae Baird, 1845

Genus *Cypris* O. F. Müller, 1776

Diagnosis (after Martens 1990, Meisch 2000 and Karanovic 2012). Valves with the anterior selvage largely displaced inwards. LV anteriorly with a well-developed inner list. LV with an additional inner list. RV with a lip-like anteroventral margin. LV overlapping the RV posteriorly and ventrally. Walking leg (L6) 4-segmented; basal segment (protopod) with a d_1 -seta *c.* 2–3x as long as the d_2 setae. Caudal rami slender.

Distribution. Distributed worldwide, but highest species richness found in the Afrotropical and Oriental regions (Meisch *et al.* 2019).

***Cypris pretusi* Mesquita-Joanes, Aguilar-Alberola, Palero & Rueda sp. nov.**

(Figs. 5–7)

Type locality. Rambla de Orduña, a tributary of Palància river, El Toro municipality, Castelló Province, Spain (Table 1).

Type material. Holotype: A female from Rambla Orduña, with soft parts mounted on a microscope slide in Hydromatrix®. Valves, coated for SEM, stored in a micropalaeontological slide (MUVHNZY0005).

Paratypes: Three females (MUVHNZY0006, MUVHNZY0007, MUVHNZY0008) from Bassa s'Enclusa, Minorca. Soft parts mounted on microscope slides in either Hydromatrix® or glycerin, and valves stored dry in micropalaeontological slides. Three more females from Bassa s'Enclusa *in toto* in ethanol in a small vial (MUVHNZY0009), and other three females *in toto* from R. Orduña in a micropaleontological slide (MUVHNZY0010).

Repository: The holotype and all paratypes are deposited in the Museum of Natural History of the University of Valencia (MUVHN), Burjassot, Spain. Other individuals from the same sites as the holotype and paratypes and from additional sites (Table 1) are stored in the ostracod collection of F. Mesquita at the “Cavanilles” Institute of Biodiversity and Evolutionary Biology of the University of Valencia, Paterna, Spain.

Derivation of name. Specific epithet dedicated to Dr. Joan Lluís Pretus (Dep. Ecology, Univ. of Barcelona), who collected the samples from s'Albufera des Grau. We dedicate this species to him, not only because of providing the samples, but also for his dedication to the knowledge and preservation of aquatic environments and their inhabitants.

Abbreviated diagnosis. Intermediate-sized (~1.9 mm long) species of the genus *Cypris*. Cp subovate in dorsal view, relatively elongated and slender compared to other members of the genus. Posterior margin of valves with a row of small subequal denticles. Frontal edge of the Cp beak-shaped, without conspicuous spines. Swimming setae on the An2 not reaching the tips of the terminal claws.

Description of adult female (males unknown). Cp (Fig. 5, Table 2): L c. 1.7–2.0 mm. RV front edge protruding and embracing LV, producing in dorsal view a beak-like shape. LV anteriorly conspicuously shorter than RV. Each valve postero-ventrally with a row of small pointed denticles on their external margin. These denticles are larger and

less numerous in the RV, and smaller but in higher numbers in the LV, although they can be difficult to observe or almost absent on the LV. Both valves with a distinct postero-dorsal angle in lateral view. Largest H located slightly in front of mid-length. Valves widest just before or at mid-length and covered with short setae. External surface of valves with small, reticulated-like pits, more conspicuous near the valve margins. Selvage inwardly displaced from the valve margin in the RV, both at the posterior and anterior parts, only slightly but variably displaced at the anterior part of the LV, and peripherally located at its posterior part.

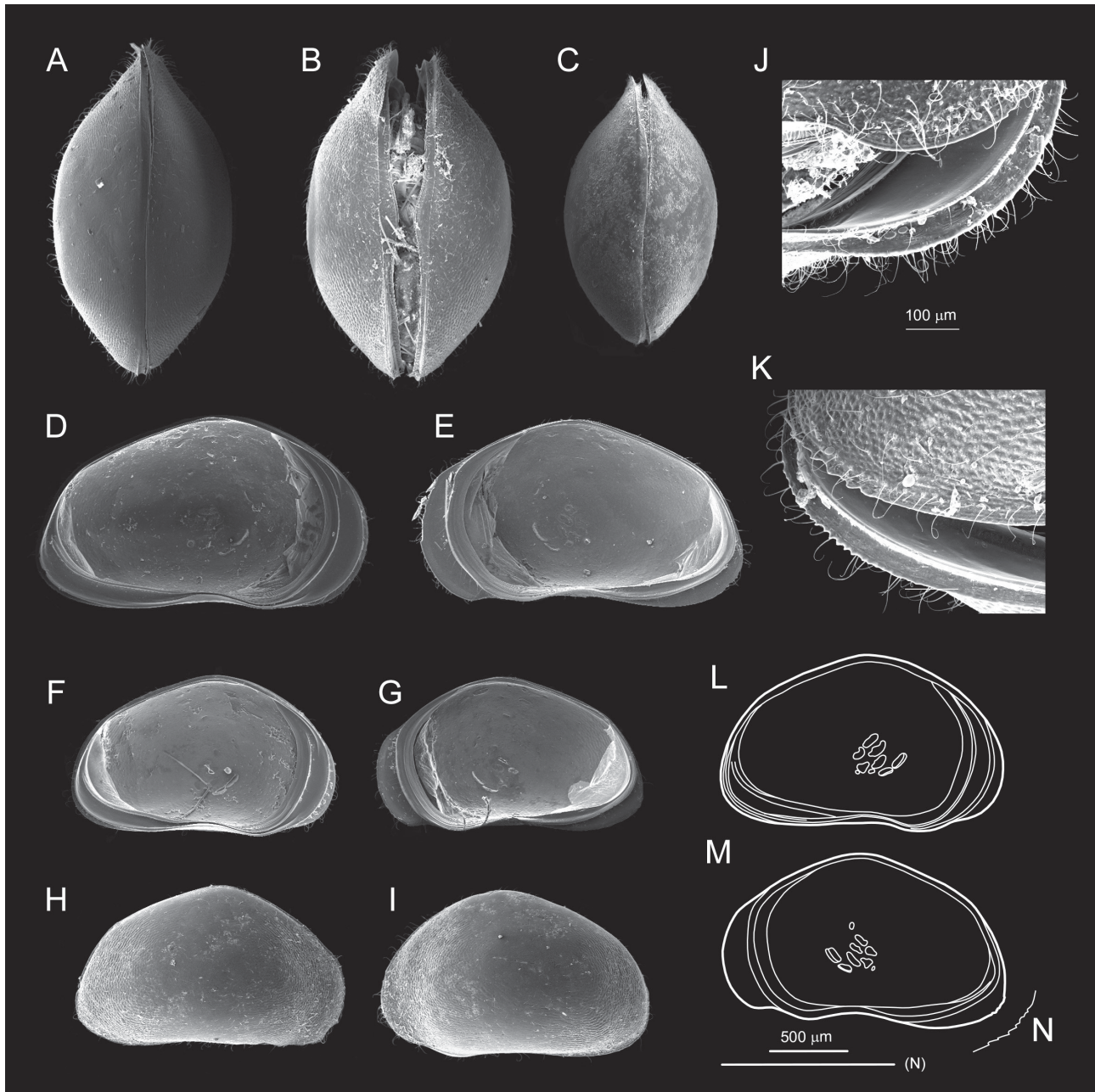


FIGURE 5. Scanning electron micrographs (A-K) and drawings (L-N) of shells and valves of *Cypris pretusi* sp. nov. A-B: dorsal (A) and ventral (B) view of whole specimens from R. Palància (A: MUVHNZY0000, B: MUVHNZY0001). C: dorsal view of a specimen from Minorca (MUVHNZY0002). D-E: Inner view of left (D) and right (E) valves of specimen MUVHNZY0005 from R. Palància. F-G: Inner views of left (F) and right (G) valves of a specimen MUVHNZY0003 from Minorca. H-I: Outer views of right (H) and left (I) valves of a specimen MUVHNZY0004 from Minorca. J: detail of anteroventral part of a female (MUVHNZY0001). K: detail of postero-ventral part of a female (MUVHNZY0001) showing the apparent denticles, larger and less numerous in the right valve. L-N: drawings of left (L) and right (M) valve, and detail of the posterior edge of right valve (N) of a specimen (MUVHNZY0008) from Minorca. Small scale below M=500 µm for A-I, L and M, larger scale=500 µm for N. Scale for J-K indicated as=100 µm.

TABLE 2. Measurements of minimum (Min), maximum (Max) and mean carapace length and height for adult females and A-1 juveniles of *Cypris pretusi* **sp. nov.** for each of the two study areas where the species has been found. N= number of individuals measured.

		LENGTH (mm)				HEIGHT (mm)			
		Min	Mean	Max	(N)	Min	Mean	Max	(N)
Minorca	Adult fem.	1.62	1.74	1.84	23	0.92	1.02	1.1	17
	A-1 juv	-	1.30	-	1	-	0.76	-	1
Palància	Adult fem.	1.94	2.04	2.12	22	1.08	1.15	1.22	18
	A-1 juv	-	1.50	-	1	-	1.10	-	1

An1 (Fig. 6) very similar to that of the type species of the genus. Rome organ small and bottle-shaped on the ventral edge of the second articulated segment. Dorsal seta on the third segment hirsute. Y_a one third of its length longer than the shortest seta on the last (seventh) segment.

An2 natatory setae (Fig. 6) not reaching the tips of the terminal claws. Longest seta of the exopod almost as long as the corresponding endopod segment, reaching the base of the set of swimming setae.

Md-p (Fig. 6) with four subapical setae on the outer edge of the third podomere. The branchial plate (exopod) has six relatively weak setae of unequal length.

Mx palp (Fig. 7) with distal podomere rectangular, more than 2x as long as wide; the first podomere with 5 apical plus 2 dorsal subapical setae, one of the dorsal subapical setae about half as long as the others. Third endite with two serrated *Zahnborsten*. Exopod (branchial plate) with 19+6 setae.

L5 (Fig. 7) with endopod (palp) carrying three apical setae of unequal length (1 short, 1 long, 1 intermediate); respiratory plate with 5 long and 1 short rays; protopod with 2 *a*-setae, 1 *b* and one *d*-seta, apart from an apical brush of c. 13 subequal setae plus a shorter one closer to the *d*-seta.

L6 (walking leg; Fig. 7) with d_1 seta about 2x as long as d_2 . *f*-seta reaching the distal edge of the last segment. Seta h_1 thin and smooth, not claw-like.

L7 (cleaning leg; Fig. 7) without special features, similar to type species.

CR slender, with s_a seta more than half the length of the s_p seta. Claws thin and long, almost completely straight. Attachment distally bifurcated.

Interpopulation variability. Differences in Cp size between populations and instars are shown in Table 2. Cp L and H larger in the continental than in the island population. Anterior selvage of LV not largely displaced inwardly in the specimens from Palància river (Fig. 5), deviating from the diagnosis of the genus; more clearly displaced in the Minorca specimens.

Ecology and distribution. The species is only known from two areas, one on the Eastern Iberian Peninsula, and the other in the island of Minorca. In the first case, it was found in two headstreams and the main channel of River Palància, a relatively small Mediterranean river suffering strong variations in flow discharge between seasons, some of its headstreams drying out in summer. In Minorca, the species was collected from a temporary pond in the Natural Park s'Albufera des Grau. Table 1 provides a summary of main characteristics of the sites and their environment.

Differential diagnosis. The most similar species to *C. pretusi* **sp. nov.** is the type species of the genus *C. pubera*. Even though *C. pubera* attains a slightly larger size, both have a very similar internal structure of the valves. They differ however in the presence in *C. pubera* of some larger spines on the posterior edge of the valves, plus a row of conspicuous denticles in the anterior part. On the other hand, *C. pretusi* **sp. nov.** has no apparent or just tiny denticles in the anterior part, and those at the posterior edge are all small and subequal. Furthermore, in *C. pubera* both valves have a similar non beak-like shape at their frontal end (although it can show slightly beak-shaped carapaces; Martens 1990: Fig. 4C), but are asymmetrical and more clearly beak-like in *C. pretusi* **sp. nov.** This character makes the new species similar to *C. decaryi*, but this species has a higher W:L and H:L ratio than *C. pretusi* **sp. nov.** (W:L=0.69-0.77 in *C. decaryi* vs. 0.55-0.56 in *C. pretusi* **sp. nov.**; H:L=0.61-0.67 in *C. decaryi* vs. 0.53-0.61 in *C. pretusi* **sp. nov.**; *C. decaryi* data from Martens, 1990). In addition, the postero-ventral calcified inner lamella of LV and the postero-ventral fused zone of RV are wider in *C. pretusi* **sp. nov.** than in *C. decaryi*. The soft part anatomy of *C. pretusi* **sp. nov.** is very similar to *C. pubera*, but the swimming setae on the second antennae are a bit shorter (not reaching the tips of the terminal claws) in the new species.

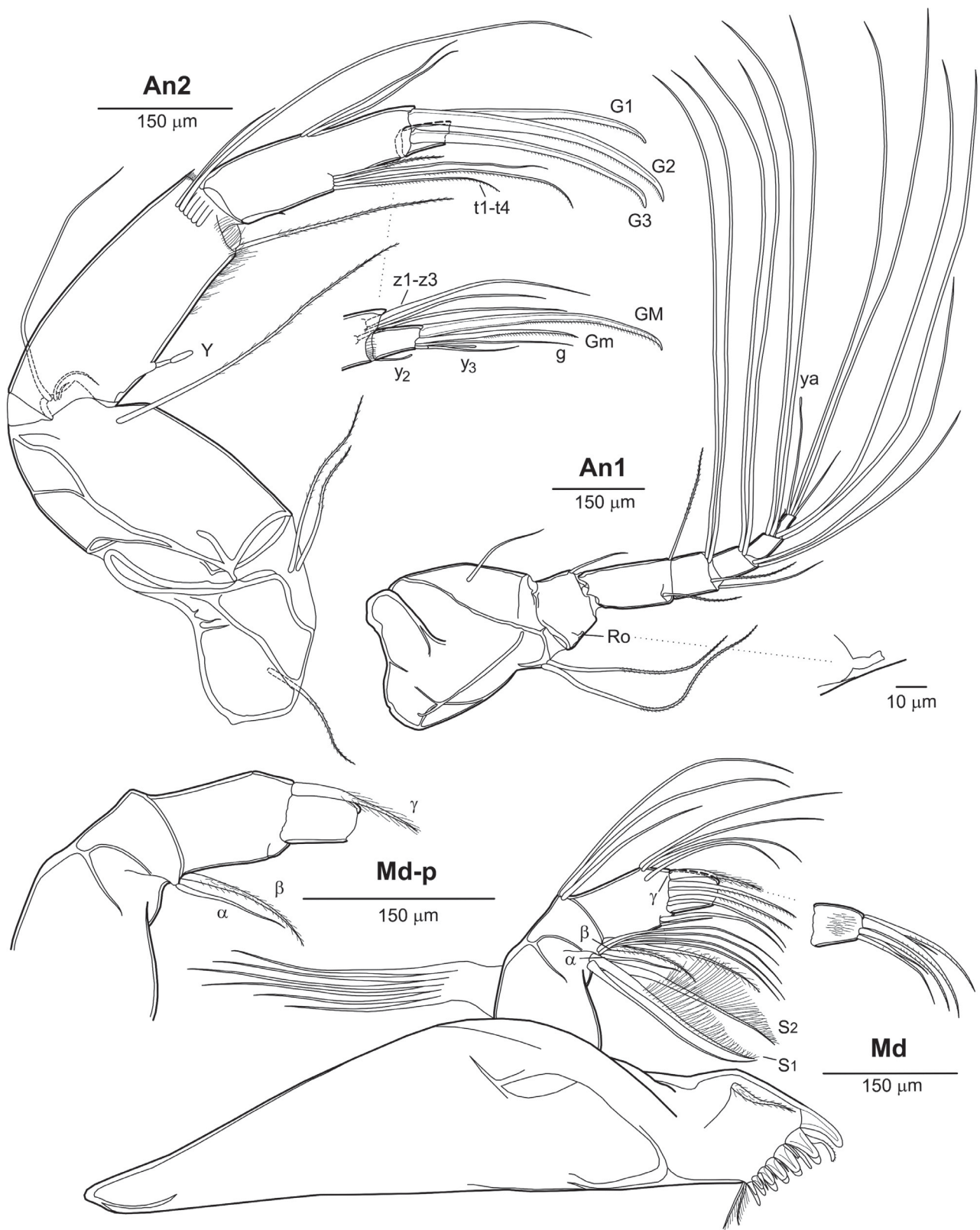


FIGURE 6. Details of the antennula (An1), antenna (An2), and mandibula (Md) of *C. pretusi* **sp. nov.** All drawings from individual MUVHNZY0005, except branchial plate of Md and Ro (MUVHNZY0006).

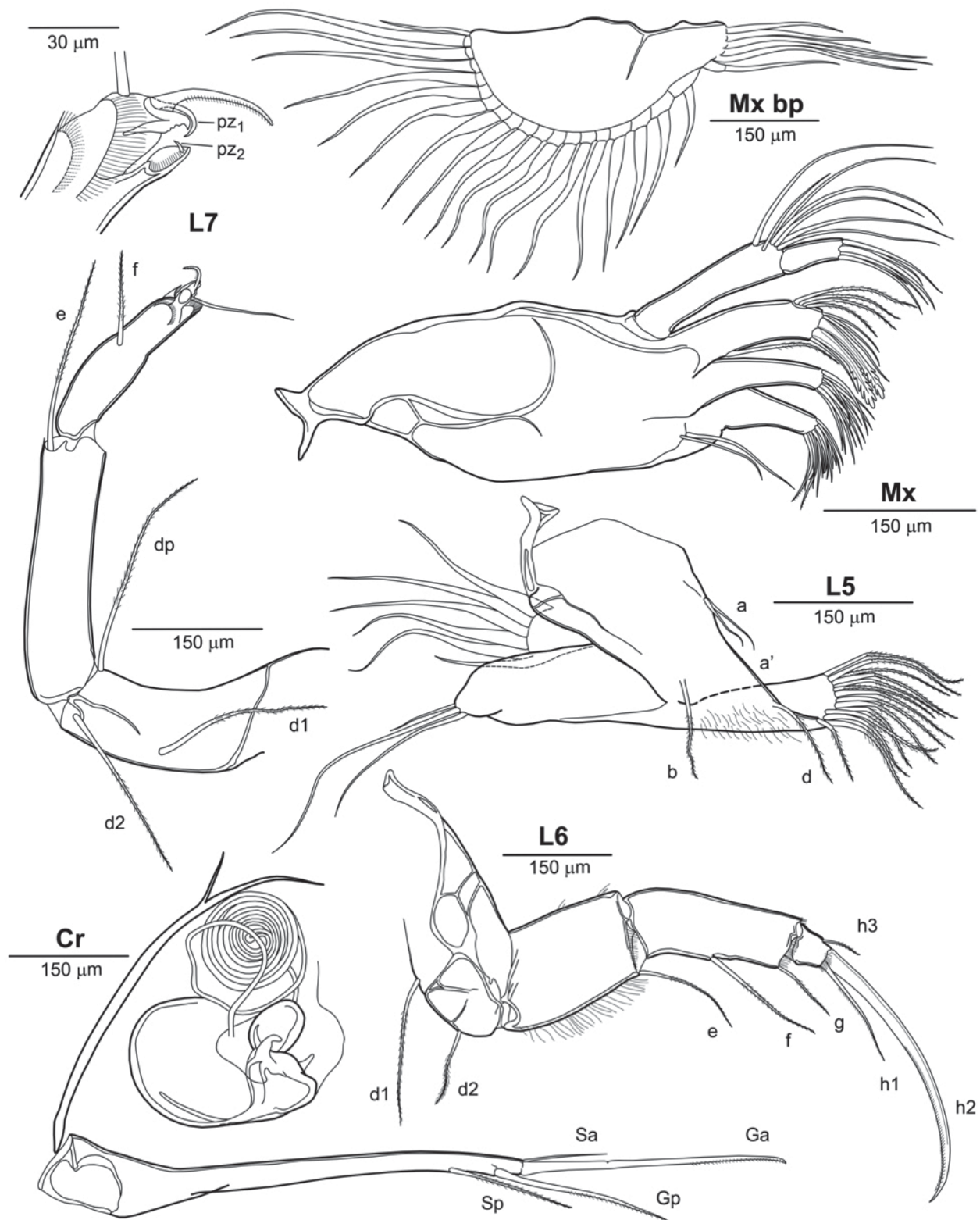


FIGURE 7. Details of the maxillula (Mx), maxillular branchial plate (Mx bp), 5th limb (L5; maxilliped), 6th limb (L6; walking leg), 7th limb (L7; cleaning leg) and its pincer and caudal ramus (Cr), together with the female genital organ of *C. pretusi* **sp. nov.** All drawings from individual MUVHNZY0005, except branchial plates of Mx and L5, detail of the tip of L7 (MUVHNZY0006) and female genital organ (MUVHNZY0007).

Phylogenetic analysis

The new COX1 sequences obtained for *Cypris pubera*, *Cypris pretusi* **sp. nov.** and *Cypris bispinosa* have been uploaded to Genbank under accession numbers MT024244, MT024245 and MT024246 respectively. The final alignment of DNA sequences obtained from the *Cypris* specimens and previously available Genbank data included 732 bp positions. The DNA substitution model selected according to the AICc method was the Hasegawa-Kishino-Yano model (HKY) with 57.92% of the sites being evolutionarily invariable. The phylogenetic tree obtained by Maximum Likelihood (Ln=-2967.47) strongly supported the clustering of *C. pretusi* **sp. nov.** and *C. bispinosa* and suggests the presence of species-level differences between *Cypris pubera* specimens (Fig. 8). As expected, K2P genetic distances were slightly larger than average p-distances in all cases, since they can take into account multiple substitutions per site. Both p-distances and K2P distances between *Cypris pretusi* **sp. nov.** and other *Cypris* species (e.g. K2P distances between *C. pretusi* **sp. nov.**/*C. bispinosa*: 0.214±0.015) were similar to those observed between *Strandesia obtusata* (Sars, 1901) and *Strandesia velhoi* Higuti & Martens, 2013 (Higuti *et al.*, 2013) (K2P=0.221±0.018) and between different *Cypris* species (Table 3).

TABLE 3. Estimates of genetic distances over sequence pairs between taxa and corresponding standard error estimates obtained by a bootstrap procedure (500 replicates). The number of observed base substitutions per site (p-distance) are shown above the diagonal and those estimated under the K2P model are shown below the diagonal.

	<i>Strandesia obtusata</i>	<i>Strandesia velhoi</i>	<i>Cypris triaculeata</i>	<i>Cypris pubera</i>	<i>Cypris pretusi</i> sp. nov.	<i>Cypris bispinosa</i>
<i>Strandesia obtusata</i>		0.189±0.014	0.220±0.016	0.207±0.016	0.232±0.016	0.237±0.017
<i>Strandesia velhoi</i>	0.221±0.018		0.206±0.016	0.200±0.015	0.222±0.016	0.230±0.016
<i>Cypris triaculeata</i>	0.264±0.024	0.244±0.024		0.185±0.014	0.198±0.014	0.217±0.016
<i>Cypris pubera</i>	0.246±0.022	0.236±0.021	0.217±0.020		0.182±0.016	0.202±0.016
<i>Cypris pretusi</i> sp. nov.	0.283±0.024	0.266±0.024	0.232±0.020	0.211±0.022		0.184±0.015
<i>Cypris bispinosa</i>	0.290±0.026	0.280±0.026	0.258±0.025	0.238±0.023	0.214±0.021	

Key to species of the genus *Cypris* found in Europe (modified after Martens 1990; Meisch 2000; Fuhrmann 2012)

- 1a Valves in dorsal view each with a large, lateral spine *C. bispinosa*
- 1b No large lateral spines 2
- 2a Surface of valves densely covered with longitudinal lists *C. striata*
- 2b No longitudinal lists 3
- 3a Valve surface conspicuously sculptured and pitted *C. granulata*
- 3b Valve surface smooth or with smaller and shallower pits 4
- 4a Frontal edge of valves with a row of spines. Right valve margin caudally with 3-5 spines, one of them conspicuously larger than the others 5
- 4b No conspicuous spines posteriorly nor anteriorly. Valve frontal edges asymmetrical; right valve clearly lip-like, showing a beak-like frontal part in dorsal view. 6
- 5a Largest posterior spine on the right valve located near the ventral edge *C. pubera*
- 5b Largest posterior spine on the right valve located near the point at maximum carapace length on the posterior edge *C. triaculeata*
- 6a Carapace slightly elongated in dorsal view, and dorsally weakly arched in lateral view. Postero-ventral fused zone wide. Posterior edge of valves faintly serrated, i.e. with a row of subequal denticles (Fig. 5k) *C. pretusi* **sp. nov.**
- 6b Carapace globose in dorsal view and dorsally strongly arched in lateral view. Postero-ventral fused zone narrow. Posterior edge of valves smooth *C. decaryi*

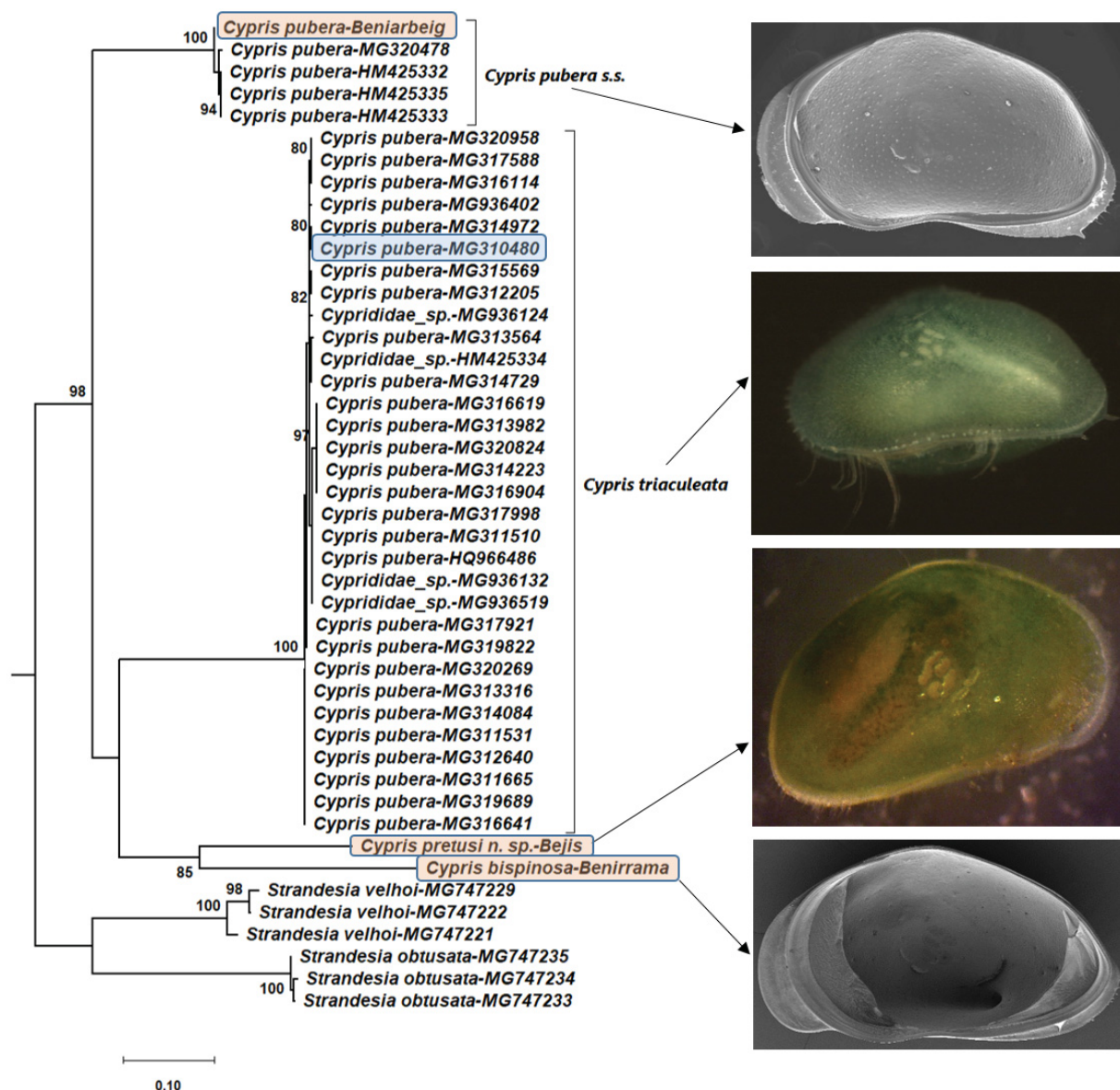


FIGURE 8. Maximum Likelihood tree inferred using the Hasegawa-Kishino-Yano model on the COX1 alignment data. Bootstrap support values (percentage of trees in which the associated taxa clustered together) larger than 80 are shown next to the branches. The tree is drawn to scale, with branch lengths measured in the number of substitutions per site. Pictures: *C. pubera*, SEM figure of RV (inner view) from R. Ebo (Zamora *et al.* 2005b); *C. triaculeata*, photograph of individual BOLD:SAOST070-08.COI-5P (Genbank code MG310480); *C. pretusi* sp. nov., individual from Bejis (sampled 15th of March 2019; Table 1); *C. bispinosa*, individual from Benirrama pond (mud culture, June 2019).

Discussion

Relationships to other species. *Cypris pretusi* sp. nov. comes morphologically very close to *C. pubera* and its allied species *Cypris triaculeata*, although the latter has been considered an uncertain species by some authors (Martens *et al.* 2013; Meisch *et al.* 2019) (but see Fuhrmann 2012 and discussion below). Although their soft parts and inner carapace structure are very similar, the new species can be distinguished from these two species by the lack of conspicuous spines in the front edge of valves, the absence of large spines on its posterior edge, and by its beak-shaped frontal end of the carapace in dorsal view. This latter feature may allow confusion with other similar species, such as *C. decaryi* and *Cypris galefensis* Martens 1990, but *C. pretusi* sp. nov. is slenderer, has a posterior row of denticles on the edge of each valve and has a different inner valve structure, including wider fused zones. The

new species has four subapical setae on the outer edge of the third podomere of the mandibular palp, while Martens (1990; Fig. 1A) figures only three in *C. pubera*. Also Neale (1976a; 1976b) drew three setae in *C. decaryi*, but four in *C. granulata*. However, we observed four setae in *C. decaryi*, *C. bispinosa* and *C. pubera* in the individuals we examined so far, not only in *C. pretusi* **sp. nov.**, so this does not seem to be a valid specific differential character. Also four setae are found in adults of species from close genera, such as *Eucypris virens* (Jurine, 1820) and *Heterocypris bosniaca* Petkowski, Scharf and Keyser, 2000, as shown in Smith & Martens (2000) and Aguilar-Alberola & Mesquita-Joanes (2013), although only three setae are present in A-1 juveniles of those two species. It might be the case that adult specimens of all *Cypris* species have four subapical setae on the outer edge of the third podomere of the mandibular palp, and therefore those drawings in the literature with just three setae might correspond to juveniles or specimens that have one seta accidentally missing.

Another species that comes close to *C. pretusi* **sp. nov.** is *Cypris maculosa*. This is considered to be a synonym of *C. pubera* in some publications (Yu *et al.* 2009; Karanovic 2012; Martens *et al.* 2013), while it is kept as a separate species in others (Smith *et al.* 2018; Meisch *et al.* 2019). We agree that they are not the same species, mostly because of the lack of spines on the edge of the carapace in *C. maculosa*. Furthermore, the figures in the original description of specimens from the northern Caucasus by Bronstein (1925) show a carapace in dorsal view that resembles *C. decaryi* rather than *C. pubera*, because of a wider carapace and frontal beak shape. Also the drawings and pictures presented by Okubo (1990; 2004) for this species resemble the original description of *C. maculosa* by Bronstein (1925), and are also similar to *C. decaryi*, but not to *C. pubera*. Strangely enough, Bronstein (1928) shows lateral views of *C. maculosa* from another locality that are rather similar to *C. pretusi* **sp. nov.** (unlike the drawing in Bronstein (1925), which resembles *C. decaryi*). Maybe the later figures made some authors (Yu *et al.* 2009; Martens *et al.* 2013; Rasouli *et al.*, 2016) synonymize *C. maculosa* with *C. pubera*, although no posterior spines are observed; in our view, future studies should investigate whether *C. decaryi* might actually be a junior synonym of *C. maculosa*. If the specimens shown in Bronstein (1928) turn out to belong to the same species as the one described in the present survey (lack of conspicuous spines, wide fused posterior zone, valve inner structure), then the biogeographic distribution of *C. pretusi* **sp. nov.** would be much wider than discussed herein.

The COX1 gene has been suggested to be an informative molecular marker at several taxonomic scales, but particularly at the species level. The phylogenetically closest species to *C. pretusi* **sp. nov.** is *C. bispinosa*, according to our molecular results. The soft parts of *C. pretusi* **sp. nov.** (e.g. inner structure of the valves' edges) are very similar to those of *C. bispinosa*, but they can be easily distinguished by the large lateral spines in the latter. The most similar external morphology of *C. pretusi* **sp. nov.** to *C. pubera*, rather than *C. bispinosa*, makes our phylogenetic result unexpected. Nevertheless, the clustering of *C. pubera* specimens from Eastern Spain with other *C. pubera* from Canada, and *C. pretusi* **sp. nov.** with *C. bispinosa*, together with morphological information from soft parts (see above), reinforces the status of *C. pretusi* **sp. nov.** as a valid species different from *C. pubera*. Furthermore, specimens from Canada initially assigned to *C. pubera* in the BOLD database, can be distinguished as two different phylogenetic clades. One clade includes specimens with a strong posterior spine on the right valve (see Fig. 8, corresponding to specimen BOLD:SAOST070-08.COI-5P), and the other, including the *C. pubera* analyzed here together with specimens from Canada, have a smaller postero-ventral spine. These two distinct lineages may correspond in fact to *C. triaculeata* and *C. pubera* respectively, *sensu* Fuhrmann (2012) and Fuhrmann & Goth (2011). Both morphotypes, one with a smaller postero-ventral spine (i.e. *C. pubera* s. s.) and another with a larger posterior spine (i.e. *C. triaculeata*) can be seen in published pictures from Canadian samples; Delorme (1970; 2001) shows individuals closer to *C. triaculeata*, while those shown by Smith & Delorme (2010) and Smith & Horne (2016) have a posterior set of spines closer to the typical shape of *C. pubera*, such as those shown in Müller (1900) or Meisch (2000), which match the *C. pubera* specimens analysed in the present survey. Although it was Daday (1892) who first named several varieties regarding the number of posterior spines in *C. pubera*, including the variety *triaculeata* (see discussion in Meisch *et al.* 2019), the first drawings illustrating two varieties of *C. pubera* are by Vávra (1891, Fig. 30). Our phylogenetic analysis suggests that indeed two different clades of *C. pubera* exist, and most probably one of these actually corresponds to a different species, namely *C. triaculeata*, as suggested by Fuhrmann & Pietrzeniuk (1990), Griffiths *et al.* (1998), Fuhrmann & Goth (2011) and Fuhrmann (2012), whom kept *C. triaculeata* separated from *C. pubera*. This was also the case in the checklists by Martens & Savatnalinton (2011) and Martens *et al.* (2013), but not in the most recent list by Meisch *et al.* (2019). The confusion between *C. pubera* and *C. triaculeata* may have stemmed from differences in the earlier ostracod monographs showing either one of the morphotypes or the other as *C. pubera*, disregarding the initial differentiation of two varieties by Vávra (1891). So,

we can see drawings and pictures corresponding to what we consider *C. pubera* s. s. in Müller (1900), Gauthier (1928), Klie (1938) (after Müller, 1900), and more recently in Meisch (2000), but a posterior shape of the right valve corresponding to what we consider *C. triaculeata* (but indicated therein as *C. pubera*) in the works by Sars (1928), Delorme (1970) and Sywula (1974).

Distribution and origin of *Cypris pretusi* sp. nov. At present, notwithstanding the comment about *C. maculosa* in the previous subsection, the only known distribution of *C. pretusi* sp. nov. corresponds to restricted areas of the Eastern Iberian Peninsula and Minorca (Fig. 9). However, most *Cypris* species seem to be spread over the Afrotropical and Oriental regions, and it is also in those areas where some males have been found for species with geographical parthenogenesis, such as *C. pubera* and *C. decaryi* (Martens 1990; Meisch 2000; Karanovic 2012; Martens *et al.* 2013). Bisexual populations of *C. bispinosa* are known from Northern Africa and the Iberian Peninsula (Meisch 2000), where this species shows the widest distribution (Fig. 9). In contrast, the most common *C. pubera* is distributed more widely in central, northern and eastern Europe (Fig. 9). It is also widespread in Canada (Delorme 1970) and has been found in South America (Ramón Mercau *et al.*, 2012), China (Yu *et al.*, 2009) and New Zealand (Eagar, 1994). Its sister species *C. triaculeata* is mostly known from central Europe (and Canada, according to the COX1 tree), while European populations of *C. granulata* and *C. decaryi* have only been found in or near rice fields in Macedonia and Spain (Petkovski 1964; Valls *et al.* 2014; this work), suggesting an exotic origin. Taking this into account, and the Linnean and Wallacean shortfalls (Hortal *et al.* 2015), we would not be surprised if further populations of *C. pretusi* sp. nov. are to be found in Africa or Asia in the future, wherefrom they might have colonized the Western Mediterranean, probably via migratory birds or human transport (see e.g. Horne & Smith 2004; Valls *et al.* 2016; 2017). Nevertheless, the differences in average size of adult individuals and in the internal structure of the front edge of the left valve between populations in the island of Minorca and the continent, suggest that these populations might have been isolated for a relatively long time (or that they come from different origins), pointing to long-term isolation. These populations could then represent relicts of warmer periods, but their origin from the Afrotropical or Oriental regions should be confirmed in the future.

Further discussion on *Cypris* historical taxonomy, and conclusions. As mentioned earlier in the Introduction section, the genus *Cypris* was erected by O.F. Müller to accommodate the bivalved *Monoculus* species described by Linnæus. Thus, the first ostracod species to be named using the Linnean binomial nomenclature, i.e. *Monoculus concha pedata*, later called *Monoculus conchaceus*, became *Cypris pubera*, a fact that has not been clearly recorded or highlighted in recent ostracod monographs (e.g. Meisch 2000; Karanovic 2012). This is probably due in part to some early confusions or disagreements with Müller, such as the work by Jurine (1820: p. 171) who kept *C. pubera* and *M. conchaceus* as two separate species. But Jurine's (1820: Pl. XVIII, Figs. 1–2 and Pl. XVII, Figs. 7–8) drawings do not resemble *C. pubera* as illustrated by Müller (1785: p. 56, Tab. V, fig. 1–5). At that time, Straus (1821) provided a critical discussion on the use of the generic name *Cypris*, and strongly recommended that all species named prior to Müller (1776) should be considered as *nomen nudum*, because of the lack of appropriate descriptions. Indeed, he suggested that Müller erroneously synonymized *Cypris pubera* with *Monoculus conchaceus* because of the too general description provided by Linnæus (but see Fig. 1). According to Straus' reasoning, even if Müller (1776) says that his *C. pubera* corresponds to Linnæus' *Monoculus conchaceus*, he could not be sure. Nevertheless, considering the large size of this ostracod, its green colour (rather greyish or “cinereus” in Linnæus' (1746) description), its hairy valves (*pubera*) and its swimming behaviour, there is a high probability that it referred to the same species, because only relatively large invertebrates were being described at that time. We may indeed think of similar species (in terms of ovoid shape, large size and fast swimming behavior) commonly found in Northern Europe, such as *Eucypris virens* (Jurine, 1820) (although not so hairy), *Heterocypris incongruens* (Ramdohr, 1808) (but smaller and not greenish) or *Bradleystrandesia* spp. (but these are smaller and usually reddish or brownish). Small animals are usually less studied than bigger ones, but not only in the 18th century. Nowadays, 270 years after the Linnean system was established, we still suffer from the same problem; many small animals are still undescribed, and even some of the largest ostracods in Europe, as the new *Cypris pretusi* sp. nov., await to be discovered and described. Classical taxonomic studies are under threat (Martens & Segers 2005), and the much-needed fight against the Linnean and Wallacean shortfalls, increasing biodiversity knowledge and preservation, will not benefit from the reduction in naturalistic efforts of current scientific programs. We may never know if Müller (1776) was right in considering *Cypris pubera* as the same ostracod first described and named by Linnæus, but we must follow his early steps and continue unveiling the beautiful diversity of organisms hidden below the water surface. A necessary effort to understand how nature works and to preserve the function and beauty of this wonderful life.

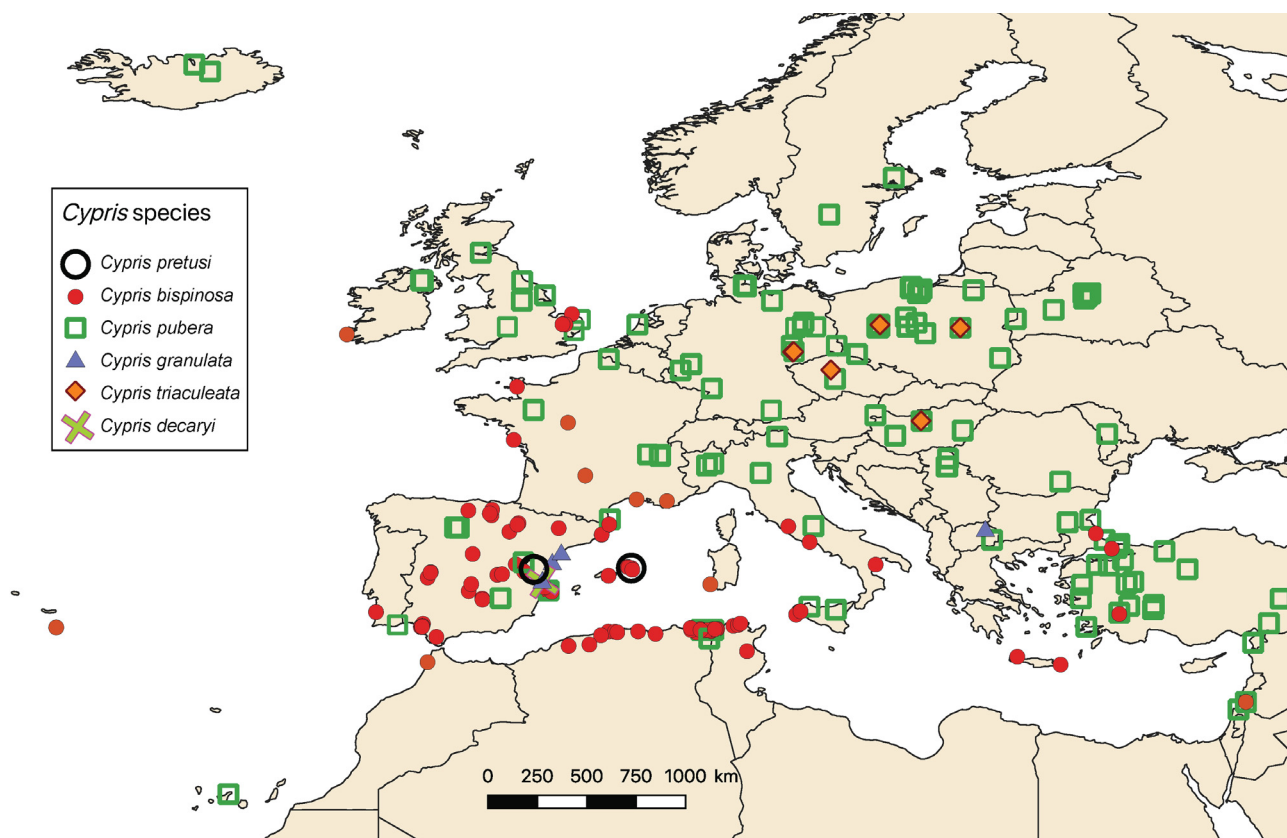


FIGURE 9. European and circum-Mediterranean distribution map of extant species of the genus *Cypris*.

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